[Frontiers in Bioscience E4, 889-896, January 1, 2012]

Epithelial-to-mesenchymal transition: possible role in meningiomas

Nives Pecina-Slaus[1,2], Tatjana Cicvara-Pecina[3], Anja Kafka[1]

[1]Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine University of Zagreb, Salata 12, HR-10000 Zagreb, Croatia, [2]Department of Biology, School of Medicine, University of Zagreb, Salata 3, HR-10000 Zagreb, Croatia, [3]Department of Radiology Clinical Hospital Dubrava, Zagreb, Croatia

TABLE OF CONTENTS

1. ABSTRACT

Epithelial-to-mesenchimal transition (EMT) is a process involved in invasion and metastasis of tumors. The occurrence of EMT during tumor progression resembles the developmental scenario and sheds light on important mechanisms for the initial step of metastasis – invasion where noninvasive tumor cells acquire motility and ultimately disseminate to distant organs. The hallmark of EMT is the loss of expression of the cell-cell adhesion molecule E-cadherin. The numerous reports by many authors as well as our own results indicate that E-cadherin plays a role in CNS tumors - meningiomas. Our studies showed that 73% of meningiomas had downregulation of E-cadherin. Moreover, loss of heterozygosity of E-cadherin was observed in 32% of meningiomas. Bound to E-cadherin in adherens junctions is beta-catenin, whose translocation to the nucleus is yet another molecular event involved in EMT. In our study beta-catenin was progressively upregulated from meningothelial to atypical, while 60% of anaplastic meningiomas showed upregulation and nuclear localization of the protein. The elucidation of molecular mechanisms that govern EMT will offer new approaches and targets to restrain metastasis.

2. INTRODUCTION

Epithelial-to-mesenchymal transition (EMT), described in 1980s, is a fascinating process vital for embryonic development, tissue remodeling, wound healing and metastasis of cancer. In normal mammalian embryonic circumstances EMT is necessary for gastrulation movements and neural crest formation. Epithelial cells that are closely held together loose their connections, acquire fibroblast resemblance and start to move individually. During this transition the nonmotile epithelial cells undergo multiple molecular changes that enable them to acquire a mesenchymal phenotype characterized by the migratory potential, changed cytoskeleton, invasive behavior and resistance to apoptosis. The fundamental event in EMT is the loss of cellular adhesion, changes of ECM (extracellular matrix) components and increased production of specific transcription factors. In order to successfully complete EMT additional molecular events are also needed which include expression of specific cell-surface proteins, reorganization of the cytoskeleton and production of ECM degrading enzymes (1). Mesenchymal cells remodel ECM through the production of matrix metalloproteinases (MMPs) a family of proteins that degrade extracellular

matrix and surface proteins, leading to the release of promigratory factors (2). Elevated levels of MMP-2, MMP-3 and MMP-9 are detected during EMT. In addition of all above mentioned molecular interactions new research reports on the involvement of specific microRNAs (3) in the regulation of the EMT program.

In the patterns of developmental biology some cells are plastic and able to perform back and forth epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions. Thus the mesenchymal cells having migrated to their target destination may revert to their original epithelial phenotype through a process known as mesenchymal-epithelial transition or MET (4). This process is even less understood than EMT, but the potential to revert to epithelial phenotype is tantalizing idea for inducing this mechanism in the therapeutic objectives.

Epithelial-to-mesenchymal transition is a process very much involved in invasion and metastasis of tumors. The occurrence of EMT during tumor progression resembles the developmental scenario and sheds light on important mechanisms for the initial step of metastasis – invasion where noninvasive tumor cells acquire motility and ultimately disseminate to organs distant from the primary site. The formation of mobile cells with metastatic potential is the result of multiple consecutive genetic changes that accumulate and represent a critical factor in tumor progression. It has been shown that genes implicated in EMT during development also control processes of tumor progression (5, 6). Therefore, identification of genes and proteins involved in EMT will improve the understanding of tumor progression and metastasis.

During invasion tumor cells loose cell-cell adhesion, gain mobility and leave the site of the primary tumor to invade adjacent tissues. This first step of metastatic cascade, invasion, is critical and crucial for all the following steps, i. e. intravasation, extravasation and metastatic colonization (6). It is believed nowadays that once the cell undergoes EMT and invades its surroundings, the remaining steps of metastasis are performed with much more ease. Therefore the great interest for EMT in current biomedical science is understandable.

The most prominent feature of EMT is the loss of expression of the cell-cell adhesion molecule E-cadherin (7). Another hallmark of EMT is the so called “cadherin switch” in which epithelial protein markers such as E-cadherin, alpha-catenin, beta-catenin and claudins are lost and replaced by mesenchymal markers – N-cadherin, vimentin and fibronectin (7-9). Recent studies have shown that mesenchymal cadherins, in particular N-cadherin, enhance tumor cell motility and migration (10). N-cadherin is found primarily in neural tissues and fibroblasts where it is thought to mediate a less stable and more dynamic form of adhesion. The phenomenon of E-cadherin’s replacement by N-cadherin in tumors is regarded as a sign of invasive behavior and progression. It has also been shown that N-cadherin’s presence has stronger impact thus overruling E-cadherin’s expression.

The numerous reports by many authors as well as our own results indicate that E-cadheirn plays a role in CNS tumors - meningiomas. It is long known that meningiomas exhibit desmosomes (11), the epithelial type of cell contact, so the presence of E-cadherin in meningiomas is not unusual. Moreover, E-cadherin is considered the main cadherin type in meningiomas (12). In this review paper we aim to bring into attention the findings on how E-cadherin behaves in meningiomas and hypothesize its involvement in EMT.

3. E-CADHERIN, A PROTOTYPE ADHESION MOLECULE

As a member of a large family of genes coding for calcium-dependent cell adhesion molecules (CAMs), the cadherin glycoproteins are expressed by a variety of tissues, mediating adhesion through homotypic binding. E-cadherin, a prototype adhesion molecule is localized on the surfaces of cells in regions of cell-cell contact known as adherens junctions. Classical cadherins – E- and N-cadherins being the best characterized – play important roles in the formation of tissues during gastrulation, neurulation and organogenesis (13). The suppression of E-cadherin expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion. The absence or reduction of this protein product causes a loss of epithelial morphology and establishment of fibroblast-like features of the cells undergoing EMT. The fibroblastoid morphological changes are associated with increased motility or invasiveness. Malignant cells are characterized in general by poor intercellular adhesion, loss of the differentiated morphology and increased cellular motility. Downregulation or complete shutdowns of E-cadherin expression, mutation of the E-cadherin gene, or other mechanisms that interfere with the integrity of the adherens junctions, are prerequisite for EMT.

The human epithelial (E)-cadherin gene *CDH1* maps to chromosome 16q22.1. The gene that Berx *et al.* (14) cloned encompasses 16 exons and spans a region of ~100 kb. The exons range from 115 to 2245 bp. Further analysis of this highly conserved gene showed 15 introns ranging from 120 bp to 65 kb. The intron-exon boundaries are highly conserved and in intron 1 a 5' high-density CpG island was identified that may have a role in transcription regulation. *CDH1* encodes a 120 kDa glycoprotein with a large extra- cellular domain, a single transmembrane segment and a short cytoplasmic domain, which interacts with the actin cytoskeleton through linker molecules, alpha-beta- and gamma-catenins. On the cytoplasmic side of the membrane, a bundle of actin filaments is linked to the E-cadherin molecules *via* a protein complex in which alpha-catenin and either beta- or gamma-catenins are included. Beta- and gamma-catenins bind to a specific domain at the E-cadherin C-terminus while alpha-catenin links the bound beta- or gamma-catenin to the actin cytoskeleton. The C-terminal cytoplasmic domain of ~150 residues is highly conserved in sequence, and has been shown experimentally to regulate the cell-cell binding function of the extracellular domain of E-cadherin, possibly through interaction with the cytoskeleton (15). The juxtamembrane region of the cadherin cytoplasmic tail has been identified as a functionally active region supporting cadherin clustering

and adhesive strength. The structure of the extracellular domain of E-cadherin contains five tandem repeats with adhesive activity. This part of the molecule also has binding sites for calcium ions situated in the pockets between the repeats. Cell-cell adhesion is mediated through homotypic interactions of E-cadherin extracellular domains in a process of lateral dimerization. Parallel dimers are able to interdigitate with dimers from neighboring cells forming the points of adhesion (13).

The mechanisms that govern EMT need to establish new transcriptional programs in order to maintain the mesenchymal phenotype which necessitates the appearance of the key transcription factors. There is evidence that specific transcription factors have a role in the transcriptional repression of E-cadherin. Those factors, acting as transcriptional repressors, include zinc finger proteins of the Snail/Slug family, Twist, deltaEF1/ZEB1, SIP1/ZEB2 and the basic helix-loop-helix factor E12/E47. Interestingly all of them act early in embryogenesis and represent molecular triggers of the EMT promotion (6, 16).

Many studies have reported that carcinoma cells can acquire a mesenchymal phenotype and express mesenchymal markers. Some of the best characterized are vimentins, desmins, alpha-SMA, FSP-1, N-cadherin, fibronectin and beta-catenin's transfer to the nucleus (3). Moreover, N-cadherin, vimentin, fibronectin, Snail, Slug, Twist, Goosecoid, FOXC2, Sox 10, MMP-2, MMP-3 MMP-9, integrin alphavbeta6, ZEB2/SIP1 and LEF-1 all increase in quantity during EMT (17). On the opposite, proteins that quantities decrease during EMT are E-cadherin, desmoplakin, cytokeratin, occludin and claudins. The involved factors are used as epithelial markers for EMT (7). Actually, all the molecular changes in the activation of transcription factors and the increase or decrease in the expression of specific molecules can serve as markers for EM transition.

4. EMT AND WNT SIGNALING

Many oncogenic pathways activated by growth factors can induce EMT. Mesenchymal cells are sources of growth factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), transforming growth factor-beta (TGF-beta), src, ras, integrin, notch and wnt. Thus corresponding signaling pathways implicated in EMT induction are TGF-beta, wnt, notch, hedgehog, nuclear factor –kB (6, 18). Moreover, crosstalk among the signaling cascades of EGF/FGF, Hedgehog, TGFbeta/PMB and certainly wnt form an interconnected network.

The classical wnt pathway has a particularly tight link with EMT and it has been shown that nuclear translocation of beta-catenin can induce EMT (6, 19). One known role of beta-catenin is in cellular architecture (20) being an essential component of adherens junctions and the description of this role is given in the previous section on E-cadherin. The other role is its function as a transcription cofactor with LEF/TCF (lymphoid enhancer factor-T cell factor). The stabilization and nuclear accumulation of beta-catenin is connected to the expression of Snail 1 and Snail 2, namely wnt/beta catenin signaling pathways have been shown to activate the transcriptional repressors Snail and Slug that suppress E-cadherin expression thus inducing EMT (6).

Members of the Snail superfamily have been shown to control genes whose products function in cell movement both in metastatic process and embryonic cell migration. Snail 2 (formerly known as Slug), a zinc finger transcription factor plays a key role in regulating EMT (21). During oncogenesis, Snail 1 and Snail 2 are responsible for enabling metastasis since they directly repress transcription of E-cadherin by interaction with its promoter (21).

Wnt signaling is essential for mammalian embryogenesis (22, 23) since it acts as a regulator of the embryonic cell patterning, proliferation, differentiation, cell adhesion, cell survival and apoptosis. It is especially important in the development of the central nervous system because processes that include synaptic rearrangements require the expression of molecular components of the wnt pathway (24). The pathway regulates the normal development of the neural plate and neural tube, and later of the brain, spinal cord, and numerous sensory and motor neurons (22). In addition to neural tissues, wnt pathway is critical for sound vascular and cardiac systems development and also modulates most aspects of osteoblast physiology (23). Yet malfunctioning of this pathway in adult organism is responsible for tumorigenesis of many different tissues, including brain tumorigenesis (24-27). Actually it has been shown that constitutive activation of the wnt pathway can lead to cancer.

The wnt/wingless pathway was first discovered in mouse and *Drosophila* and is one of the most interesting signal transductions, in which key components have multiple functions. In vertebrate cells, it is named after Wnt proteins, a family of highly conserved secreted signaling molecules that regulate cell-to-cell interactions during embryogenesis. As currently understood, Wnt proteins bind to receptors of the Frizzled family on the cell surface. Through several cytoplasmic relay components, the signal is transduced to beta-catenin, the main signaling molecule of the pathway, which then enters the nucleus to activate transcription of wnt target genes (23). APC (adenomatous polyposis coli) is a critical component in the formation of multiprotein complex with axin/axin2, casein kinase I and glycogen synthase kinase 3-beta (GSK3-beta). Beta-catenin is recruited to this complex, phosphorylated, ubiquitinated and navigated to the proteasome. When wnt ligand is absent, beta-catenin is being destroyed. In response to wnt signaling, or under the circumstances of mutated components of the complex, beta-catenin is stabilized, accumulates in the cytoplasm and enters the nucleus, where it finds a partner, a member of the DNA binding protein family LEF/TCF. Target genes for beta-catenin/TCF among others encode for c-MYC, cyclin D1, c-jun, matrix metalloproteinase MMP-7 and many others (The wnt homepage http://www.stanford.edu/group/nusselab/cgi-bin/wnt/target_genes)/

Besides its role in cellular architecture, E-cadherin has a role in wnt signaling too. E-cadherin is an indirect modulator of wnt signaling. Since it binds to and sequesters cytoplasmic beta-catenin it is involved, in such a fashion, in the modulation of the signal. Beta-catenin has actually a dual role in the EMT; in adherens junctions it enhances cell-cell adhesion when bound to cadherin complexes, but his other role is as a transcriptional coactivator together with LEF/TCF. The loss of E-cadherin frees beta-catenin from its cytoplasmic tail enabling it afterwards to move to the cell nucleus and activates pro-metastatic genes.

5. E-CADHERIN IN MENINGIOMAS

Novel research including our own studies demonstrates the involvement of E-cadherin in meningiomas. Meningiomas originate from the meningeal coverings of the brain and the spinal cord and are derived from arachnoidal (meningothelial) cells. They account for approximately 25% of primary intracranial and intraspinal neoplasms. The knowledge on genetic susceptibility for meningioma has come from studies of rare genetic syndromes. Besides many other syndromes, meningiomas are a principal feature of neurofibromatosis type 2 (NF2), a rare autosomal dominant disorder caused by germline mutation in the *NF2* gene on 22q12. Loss of expression of *NF2* protein product merlin (schwannomin) is consistent finding in all NF2 associated meningiomas and in about half of sporadic cases (28, 29). Merlin behaves as a tumor suppressor protein and it has been shown to interact with proteins constituting cadherin mediated adherens junctions. In approximately 60% of sporadic meningiomas, the *NF2* gene is inactivated by a small mutation (29, 30). The remaining percent of sporadic meningioma failed to show the aberrations of neither chromosome 22, nor the mutations in the *NF2* gene. This discrepancy suggests that an alternative pathogenetic mechanism is responsible for the development of these sporadic tumors. Candidate genes include *LARGE, MN1, BAM22*, *INI* and *TP53*. Also implicated are *DAL1* and *CDKN2A,* as well as *sis, myc, ras* and *mos* oncogenes (29, 30).

In almost all human carcinomas investigated through a great number of studies the patterns of the expression of E-cadherin gene have always been consistent. The loss of expression was associated to loss of tumor differentiation, with a high grade and poor prognosis.

Based on the facts specified in the introduction, our group has decided to enlighten E-cadherin's expression in meningiomas and to investigate its protein levels. Our investigations were conducted on a collected sample of 60 meningiomas together with autologous blood tissues. All tumors were studied by pathologists and classified according to the WHO criteria (31). The majority of meningiomas in our sample corresponded to grade I of WHO classification of CNS tumors and thus were benign, slowly growing tumors (31, 32). Within the benign category our sample consisted of 14 meningothelial subtype, 10 fibrous (fibroblastic), 10 transitional (mixed), 1 psammomatous and 10 angiomatous. Meningiomas associated with less favorable clinical outcome correspond to grade II (atypical) and those who will exhibit features of malignant behavior – to grade III (anaplastic). Therefore we also collected 10 atypical and 5 anaplastic meningiomas cases.

Our immunohistochemical analysis showed that overall 73% of meningiomas had downregulation of E-cadherin expression. The expression of the protein according to meningioma grades was as follows. E-cadherin downregulation was observed in 50% of meningothelial; 80% of fibrous; 80% of transitional; 90% of angiomatous; 80% of atypical; and in 80% of anaplastic. Thirty–six percent of meningothelial and 40% of each fibrous, transitional and angiomatous showed intense lower expression, while in atypical and anaplastic intense downregulation or complete loss of the protein product was noted in 60% of each grade.

In order to verify whether E-cadherin's changes at the protein level have genetic roots in meningiomas, gross deletions or loss of heterozygosity (LOH) of the *CDH1* gene were investigated. PCR amplification of the microsatellite markers for *CDH1* gene visualized on Spreadex EL 300 gels (Elchrom Scientific, Switzerland) and on 15% polyacrylamide gels were used for LOH detection. Absence or significant decrease of one allelic band in the tumor compared with autologous blood sample was considered as LOH of *CDH1* gene (Figure 1B).

We found 32% of meningioma samples with LOH of the *CDH1* gene. When we distributed total E-cadherin's changes to specific tumor types, changes were observed in 27% of meningothelial meningiomas, 67% of fibrous, 33% of transitional (mixed) and 75% of angiomatous. The changes observed at the genetic level were consistent to the oscillations of the expressed E-cadherin protein. Fifty-six percent of samples with LOH were accompanied with the downregulation of E-cadherin protein expression (Figure 1 D).

Since the loss of E-cadherin's expression frees beta-catenin from the intracellular part of the molecule creating an increase in the cytoplasmic pool of beta-catenin, and since its transfer to the nucleus has been regarded as one of the main events for EMT thus assigning it a mesenchymal marker role, it was important to gather data on the expression and cellular localization of the main downstream wnt signaling effector molecule, beta-catenin. Seventy five percent of our samples with genomic changes of the *CDH1* had nuclear localization of beta-catenin protein (Figure 1 E). At the same time in cases where *CDH1*'s changes were not detected the location of the beta-catenin protein was primarily in the membrane or was not detectable. The differences in the frequencies of the analyzed features were tested with the Pearson χ^2 test employing Yates correction when appropriate. Our findings demonstrated that there was significant association between the genetic changes of *CDH1* and the nuclear localization of beta-catenin protein (χ^2 =5.25, df =1, P<0.022). Statistically relevant association between decreased expression of E-cadherin and beta-catenin transfer to the

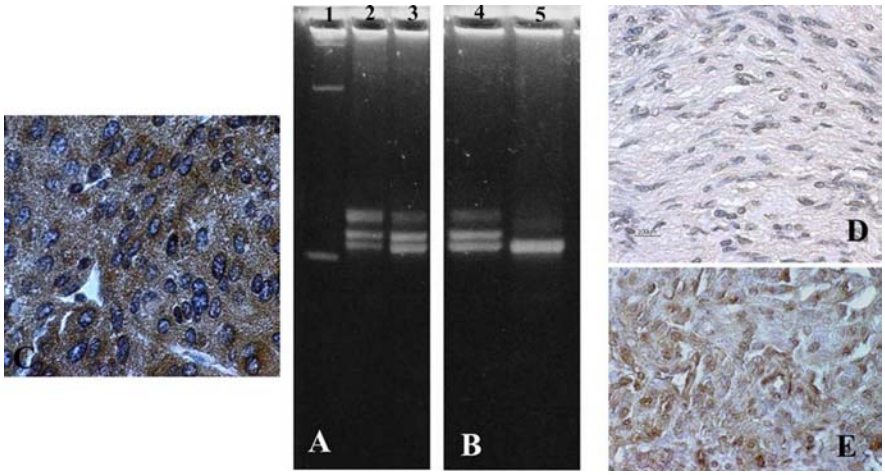

Figure 1. A. Informative meningioma sample. Lane 1– M3 standard; lane 2–informative tumor sample without LOH; lane 3– corresponding blood. B. Meningioma sample demonstrating LOH of the E-cadherin gene. Lane 4– corresponding informative blood sample; lane 5 –LOH of the E-cadherin gene. Polymorphic marker D16S752 is shown on Spreadex gels (Elchrom Scientific). C. and D. Meningioma samples immunohistochemically stained for the expression of E-cadherin proteins. Patient without LOH demonstrating strong expression (C). Patient with *CDH1* LOH demonstrating loss of expression (D) and (E) nuclear localization of beta-catenin.

nucleus could not be established, but nevertheless was noticed that 36.4% of samples with lower E-cadherin expression had beta-catenin located in the nucleus.

The relationship of beta-catenin expression to tumor grade demonstrated progressive upregulation from meningothelial (42.9%), through fibrous (50%), transitional (70%), angiomatous (80%), to atypical (90%). Upregulation of beta-catenin was noted in 60% of anaplastic cases which all had the protein localized in the nucleus.

Classifications of meningioma subtypes are sometimes doubtful with respect to prediction of patient outcome, recurrence or response to treatment. Understanding the genetic basis and molecular etiology of meningioma is essential for clinical phenotype determination as well as patient outcome. Since the loss of E-cadherin is a well known prerequisite for tumor cell invasion, the findings suggest that meningioma types that lost E-cadherin may hide future invasive behavior.

6. DISCUSSION AND FUTURE PERSPECTIVES

When reconsidering E-cadherin in human tumors, the general picture shows that the loss of E-cadherin-mediated cell adhesion correlates with the loss of the cellular morphology and with the acquisition of metastatic potential, thus naming E-cadherin a tumor invasion suppressor. Our work on E-cadherin in meningiomas demonstrates that changes of E-cadherin are frequent among meningioma types leading us to the belief that those changes are an integral part of the mechanisms of meningioma development.

Epithelial-mesenchymal conversion is an important mechanism for the initial step of metastasis and the hallmark of EMT is the loss of E-cadherin expression. Therefore our results may be indicative of EMT in meningiomas. The wnt pathway has a particularly tight link with EMT and it has been shown that nuclear translocation of beta-catenin can induce EMT (6). Our previous investigations (33) indicate involvement of yet another component of wnt signaling – APC in meningiomas. We reported on the significant association between APC genetic changes and lack of wild type protein expression or presence of mutant APC proteins. APC changes also influenced beta-catenin expression and nuclear localization, showing beta-catenin's importance in the biology of meningioma. Our previous research (34) reported on two meningiomas with heteroduplexes in exon 3 of beta-catenin, suggesting that those meningiomas harbor mutations of the beta-catenin gene. Another point in favor of EMT involvement in meningiomas comes from the findings that in some meningiomas "cadherin switch" may occur, namely, E-cadherin may be replaced by N-cadherin. As we pointed out earlier his phenomenon normally happens in epithelial-mesenchymal transition. Meningiomas need not exhibit morphological signs of malignancy, and loss of E-cadherin gene may change the situation and initiate the mechanisms of future expansion. Biologic spectrum of meningiomas is wide, heterogenic and difficult to predict. Some of the histologically benign meningiomas recur unexpectedly even after complete resection and invade surrounding tissues. Meningioma classifications (31, 32) recommend caution on benign meningioma prognosis, proposing their proliferative activity and brain invasion as important characteristics that could indicate recurrence and should be considered in diagnostics and prognosis.

There are reports on E-cadherin in meningiomas by other authors and they are in general accordance with our observations (26, 35, 36). Allelic losses of *CDH1* gene were observed in fibrous and angiomatous meningiomas.

Utsuki *et al.* (37) reported on negative E-cadherin immunostaining in all of the fibrous meningiomas they examined. Schwechheimer *et al.* (11) also found that E-cadherin's expression was absent from the majority of morphologically malignant meningiomas and that the loss of its expression was correlated with tumor dedifferentiation. Brunner *et al.* (38) found lack of E-cadherins expression in 34% of meningiomas independent of their WHO grade as well as loss of membranous and positive nuclear immunoreactivity of beta-catenin. Panagopoulos *et al.* (39) report that E-cadherin expression is present in both normal brain and arachnoid cells and that E-cadherin expression changes could not be correlated to tumor grade. Nevertheless, they observed E-cadherin expression in 59% of benign meningiomas, 67% of atypical while none of the anaplastic menigiomas expressed E-cadherin in their study. Our data on the expression according to meningioma grades demonstrate that intense downregulation of E-cadherin was noticed in tumors with grades II and III. Akat *et al.* (12) described a new type of adherens junction in human meningiomas and the human meningioma cell line HBL-52. This novel junction is closely related to classic adherens junctions but is nevertheless unique and different from other junctions. The proteins it contains, among others, include E-cadherin and beta-catenin. The majority of meningiomas the authors examined were positive for E-cadherin, but the amount of expression fell in atypical or malignant cases, in which N-cadherin replaced E-cadherin. Our results on the relationship of beta-catenin expression to tumor grade demonstrated progressive upregulation from meningothelial to atypical while 60% of anaplastic cases showed upregulation of beta-catenin and all had the protein localized in the nucleus. Wrobel *et al.* (40) reported on increased expression of beta-catenin and cyclin D1 in meningiomas they examined by microarray. Sequestration of beta-catenin in the cytoplasm is important for the preservation of epithelial features of cancer cells, and acquisition of the mesenchymal phenotype correlates with the movement of beta-catenin to the nucleus. The level of its accumulation in the nucleus correlates with susceptibility to enter into EMT and gain invasive phenotype (3).

Primary tumors may sustain high levels of the epithelial-to-mesenchymal transition phenotype, nevertheless in the corresponding metastatic deposits reversed mesenchymal-to-epithelial transition is frequently observed. Reversal of EMT process – MET is probably required to allow post EMT cells to grow and so form new colonies and distant metastases. The study by Bukholm *et al.* (41) examined the expression of E-cadherin and beta-catenin in lymph node metastases from lobular breast cancers and found the re-expression of both proteins in metastases they examined as if the adherens junctions were functionally reconstituted in metastatic deposits.

Carcinoma cells need not pass through EMT to the same extent and may or may not become fully mesenchymal. It is still not known what specific signals induce EMT in carcinoma cells, but we suspect that such signals may originate in the tumor stroma (3). Cells that lose E-cadherin may become more responsive to various growth factors which will ultimately lead to the induction of EMT. It seems that metastazing cancer cells must shed their mesenchymal phenotype via a MET during the course of secondary tumor formation (1). What will cause the cell to loose mesenchymal phenotype and once again resurrect epithelial phenotype is probably hidden in the local microenvironments of distant organs. We may speculate that the secession of the signals they received in the primary tumor contributed to MET (3). It has been shown that dissociated single carcinoma cells have reduced-to-lost or cytoplasmic expression of E-cadherin, but upon reaching the distant metastatic sites, they regain a normal membranous E-cadherin content and aggregate again in solid islands. The rapid reversibility of beta-catenin's phosphorylation and dephosphorylation could also account for the dynamic modulation of E-cadherin and the recovery of adhesive properties. Thus it seems that alternate switches of reversible EMT and MET underline invasion and growth probably regulated by environmental factors, and these processes seem to be linked to modulation of E-cadherin and beta-catenin (1).

Finally, we would like to discuss new reports that describe the regulatory link of miRNA to E-cadherin and the regulation of the EMT program. One of the families of miRNA, namely the miR-200, regulates EMT by targeting the E-cadherin repressors ZEB1 and ZEB2 (42). In a paper by Sajdam *et al.* (43) the authors evidenced that specific microRNA, miR-200a, has a direct role in meningioma growth *via* E-cadherin and wnt/beta-catenin signaling pathway. Downregulated miR-200a in meningiomas promoted tumor growth by reducing E-cadherin and activating the wnt/beta-catenin signaling pathway. A direct correlation between the downregulation of miR-200a and the upregulation of beta-catenin was demonstrated in this study. miR-200a functions as a potential tumor suppressor by inhibiting wnt/beta-catenin signaling through two complementary mechanisms: direct targeting of the beta-catenin mRNA, leading to reduced beta-catenin levels, and targeting of the mRNAs for ZEB 1 and ZEB 2 with consequent upregulation of E-cadherin levels and sequestration of beta-catenin. Moreover, a report by Ma *et al.* (44) showed that miR-9 directly targets *CDH1*, the E-cadherin encoding mRNA, leading to increased cell motility. miR-9 mediated E-cadherin downregulation results in the activation of beta-catenin signaling, which contributed to upregulated expression of the gene encoding VEGF leading to increased tumor angiogenesis. In addition, miR-21 is abundantly expressed in carcinomas and has been shown to be an EMT specific microRNA (6). TGFbeta downregulates the expression of human miR-141, miR-200a, miR-200b, miR-200c, miR-205 and miR-429, which are all targeted to ZEB1 and ZEB2 mRNAs and without the presence of the above mentioned miRNAs ZEB1 and ZEB2 are upregulated and able to repress E-cadherin (5).

Changes of *CDH1* gene and the functional consequences of the changes at the protein level strengthen our conclusions on its involvement in meningioma. Moreover, beta-catenin's behavior was in accordance to E-

cadherin changes both in the disruption of adherens junctions and wnt signaling. The association of changes to meningiomas with grades II and III suggests that it is probably connected to predisposition to progression in a subset of meningiomas.

The full spectrum of signaling agents that contribute to EMT remains unclear. All things considered EMT is a very complex event requiring the specific spatiotemporal expression of molecules, their interaction and modification of a range of cellular and extracellular factors to allow cellular motility and invasion to proceed. This process can functionally be divided to development, tissue regeneration and tumor invasion where it is similar to developmental events but with the important difference that it is uncontrolled.

7. ACKNOWLEDGEMENT

This work was supported by grant 108-1081870-1905 from Ministry of Science Sports and Education, Republic of Croatia.

8. REFERENCES

1. M. Guarino, B. Rubino and G. Ballabio: The role of EMT in cancer pathology. *Pathology* 39, 305-318 (2007)

2. A. M. Eastham, H. Spencer, F. Soncin, S. Ritson, C. L. R. Merry, P. L. Stern and C. M. Ward: Epithelial-mesenchymal transition events during human embryonic stem cell differentiation. *Cancer Res* 67, 11254-11262 (2007)

3. R. Kalluri and R. A. Weinberg: The basics of epithelial-mesenchymal transition. *J Clin Invest* 119, 1420-1428 (2009)

4. D. C. Radisky: Epithelial-mesenchymal transition. *J Cell Sci* 118, 4325-4326 (2005)

5. Y. Katoh and M. Katoh: Hedgehog signaling, epithelial-to-mesenchymal transition and miRNA (Review). *Int J Mol Med* 22, 271-275 (2008)

6. Y. Wu and B. P. Zhou: New insights of epithelial-mesenchymal transition in cancer metastasis. *Acta Biochim Biophy Sin* 40, 643-648 (2008)

7. F. Lehembre, M. Yilmaz, A. Wicki, T. Schomber, K. Strittmatter, D. Ziegler, A. Kren, P. Went, P. W. B. Derksen, A. Berns, J. Jonkers and G. Christofori: NCAM-induced focal adhesion assembly: a functional switch upon loss of E-cadherin. *EMBO J* 27, 2603-2615 (2008)

8. R. B. Hazan, R. Qiao, R. Keren, I. Badano and K. Suyama: Cadherin switch in tumor progression. *Ann N Y Acad Sci* 1014, 155-163 (2004)

9. K. Gravdal, O. J. Halvorsen, S. A. Haukaas and L. A. Akslen: A switch from E-cadherin to N-cadherin expression indicates epithelial-to-mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res* 13, 7003-7011 (2007)

10. U. Cavallaro, B. Schaffhauser and G. Christofori: Cadherins and the tumour progression: is it all in a switch? *Canc Lett* 176, 123-128 (2002)

11. K. Schwechheimer, L. Zhou and W. Birchmeier: E-Cadherin in human brain tumours: loss of immunoreactivity in malignant meningiomas. Virchows Arch 432, 163-167 (1998)

12. K. Akat, C. K. Bleck, Y. M. Lee, U. Hasselman-Weiss and J. Kartenbeck: Characterization of a novel type of adherens junction in meningiomas and the derived cell line HBL-52. Cell Tissue Res 2008, 331, 401-412.

13. N. Pecina-Slaus: Tumor suppresor gene E-cadherin and its role in normal and malignant cells. *Cancer Cell Int* E3, 17 (2003) [http://www.cancerci.com/content/3/1/17].

14. G. Berx, K. Staes, J. van Hengel, F. Molemans, M. J. Bussemakers, A. van Bokhoven and F. van Roy: Cloning and characterization of the human invasion suppressor gene E-cadherin (*CDH1*). *Genomics* 26, 281-289 (1995)

15. W. J. Nelson: Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochem Soc Trans* 36, 149-155 (2008)

16. S. A. Mikheeva, A. M. Mikheev, A. Petit, R. Beyer, R. G. Oxford, L. Khorasani, J. P. Maxwell, C. A. Glackin, H. Wakimoto, I. Gonzales-Herrero, I. Sanchez-Garcia, I. R. Silber, P. J. Horner and R. C. Rostomily: TWIST1 promotes invasion through mesenchymal change in human glioblastoma. *Mol Cancer* 9, 194 (2010)

17. J. M. Lee, S. Dedhar, R. Kalluri and E. W. Thompson: The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 172, 973-981 (2006)

18. S. Y. Shin, O. Rath, A. Zebisch, S. M. Choo, W. Kolch and K. H. Cho: Functional roles of multiple feedback loops in extracellular signal-regulated kinase and wnt signaling pathways that regulate epithelial-mesenchymal transition. *Cancer Res* 70, 6715-6724 (2010)

19. C. D. Andl: The misregulation of cell adhesion components during tumorigenesis: Overview and commentary. *J Oncol*, pii: 174715 (2010)

20. M. Bienz: β-catenin: A pivot between cell adhesion and wnt signaling. *Curr Biol* 15, R64-R67 (2005)

21. L. A. Taneyhill, E. G. Coles and M. Bronner-Fraser: Snail2 directly represses cadherin6B during epithelial-to-mesenchymal transitions of the neural crest. *Development* 134, 1482-1490 (2007)

22. F. Li, Z. Z. Chong and K. Maiese: Winding through the WNT pathway during cellular development and demise. *Histol Histopathol* 21, 103–124 (2006)

23. N. Pecina-Slaus: Wnt signal transduction pathway and apoptosis: a review. *Cancer Cell Int* 10, 22 (2010) (http://www.cancerci.com/content/10/1/22).

24. A. Patapoutian, L. F. Reichardt: Roles of wnt proteins in neural development maintenance. *Curr Opin Neurobiol* 10, 392-399 (2010)

25. A. Koch, A. Waha, J. C. Tonn, N. Sorensen, F. Berthold, M. Wolter, J. Reifenberger, W. Hartmann, W. Friedl, G. Reifenberger, O. D. Wiestler and T. Pietsch: Somatic mutations of WNT/wingless signaling pathway components in primitive neuroectodermal tumors. *Int J Cancer* 93, 445-449 (2001)

26. S. L. Howng, C. H. Wu, T. S. Cheng, W. D. Sy, P. C. Lin, C. Wang and Y. R. Hong: Differential expression of wnt genes, beta-catenin and E-cadherin in human brain tumors. *Cancer Lett* 183, 95-101 (2002)

27. N. Pecina-Slaus, T. Nikuseva Martic, A. J. Deak, M. Zeljko and R. Hrascan: Genetic and protein changes of E-cadherin in meningiomas. *J Cancer Res Clin Oncol* 136, 695-702 (2010)

28. C. E. Fuller and A. Perry: Molecular Diagnostics in Central Nervous System Tumors. *Adv Anat Pathol* 12, 180-194 (2005)

29. B. T. Ragel and R. L. Jensen: Molecular genetics of meningiomas. *Neurosurg Focus* 119, E9 (2005)

30. M. Simon, J. P. Boström and C. Hartmann: Molecular genetics of meningiomas: from basic research to potential clinical applications. *Neurosurgery* 60, 787-798 (2007)

31. D. N. Louis, H. Ohgaki, O. D. Wiestler, W. K. Cavenee, P. C. Burger, A. Jouvet, B. W. Scheithauer and P. Kleihues: The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 114, 97-109 (2007)

32. M. J. Riemenschneider, A. Perry and G. Reifenberger: Histological classification and molecular genetics of meningiomas. *Lancet Neurol* 5, 1045-1054 (2006)

33. N. Pecina-Slaus, T. Nikuseva Martic, D. Tomas, V. Beros, M. Zeljko and H. Cupic: Meningiomas exhibit loss of heterosygosity of the APC gene. *J Neurooncol* 87, 63-70 (2008)

34. T. Nikuseva Martic, V. Beros, N. Pecina-Slaus, H. I. Pecina and F. Bulic-Jakus: Genetic changes of *CDH1*, APC and CTNNB1 found in human brain tumors. *Pathol Res Practice* 203, 779-787 (2007)

35. Y. Tohma, T. Yamashima and J. Yamashita: Immunohistochemical localization of cell adhesion molecule epithelial cadherin in human arachnoid villi and meningiomas. *Cancer Res* 52, 1981-1987 (1992)

36. G. Nagashima, T. Fujimoto, R. Suzuki, J Asai, H. Itokawa and M. Noda: Dural invasion of meningioma: a histological and immunohistochemical study. *Brain Tumor Pathol* 23, 13-17 (2006)

37. S. Utsuki, H. Oka, Y. Sato, N. Kawano, B. Tsuchiya, I. Kobayashi and K. Fujii: Invasive meningioma is associated with a low expression of E-cadherin and beta-catenin. *Clin Neuropathol* 24, 8-12 (2005)

38. E. C. Brunner, B. F. Romeike, M. Jung, N. Comtesse and E. Messe: Altered expression of beta-catenin/E-cadherin in meningiomas. *Histopathology* 49, 178-187 (2006)

39. A. T. Panagopoulos, C. L. Lancellotti, J. C. Veiga, P. H. P. de Aguiar and A. Colquhoun: Expression of cell adhesion proteins and proteins related to angiogenesis and fatty acid metabolism in benign, atypical, and anaplastic meningiomas. *J Neurooncol* 89, 73-87 (2008)

40. G. Wrobel, P. Roerig, F. Kokocinski, K. Neben, M. Hahn, G. Reifenberger and P. Lichter: Microarray-based gene expression profiling of benign, atypical and anaplastic meningiomas identified novel genes associated with meningioma progression. *Int J Cancer* 114, 249-256 (2005)

41. I. K. Bukholm, J. M. Nesland and A. L. Bùrresen-Dale: Re-expression of E-cadherin, a-catenin and b-catenin, but not of c-catenin, in metastatic tissue from breast cancer patients. *J Pathol* 190, 15-19 (2000)

42. P. A. Gregory, A. G. Bert, E. L. Paterson, S. C. Barry, A. Tsykin, G. Farshid, M. A. Vadas, Y. Khew-Goodall and G. J. Goodall: The miR-200 family and miR-205 regulate epithelial-to-mesenchymal transition by targeting ZEB1 and SIP1. *Nat Cell Biol* 10, 593-601 (2008)

43. O. Saydam, Y. Shen, T. Würdinger, O. Senol, E. Boke, M. F. James, B. A. Tannous, A. O. Stemmer-Rachamimov, M. Yi, R. M. Stephens, C. Fraefel, J. F. Gusella, A. M. Krichevsky and X. O. Breakefield: Downregulated microRNA-200a in meningiomas promotes tumor growth by reducing E-cadherin and activating the wnt/beta-catenin signaling pathway. *Mol Cell Biol* 29, 5923-5940 (2009)

44. L. Ma, J. Young, H. Prabhala, E. Pan, P. Mestdagh, D. Muth, J. Teruya-Feldstein, F. Reinhardt, T. T. Onder, S. Valastyan, F. Westermann, F. Speleman, J. Vandesompele and R. A. Weinberg: miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 12, 247-256 (2010)

Key words: Epithelial-to-Mesenchymal Transition, Meningioma, E-cadherin, *CDH1*, Wnt Signaling, Review

Send correspondence to: Anja Kafka, Laboratory of Neurooncology, Croatian Institute for Brain Research, School of Medicine University of Zagreb, Salata 12, HR-10000 Zagreb, Croatia, Tel. 385 1 46 21 140, Fax:385 1 45 90199, E-mail: anja.kafka@gmail.com

http://www.bioscience.org/current/vol4E.htm